

Glycolysis

Introduction to Glycolysis

This lesson features the key steps of glycolysis that are relevant for medicine (not biology). It reviews the pathways, regulation, and variation in different cells. **All cells perform glycolysis**, therefore the **default** in all cells is “see sugar, burn sugar.” That means all cells will have a **glucose transporter** that is highly favorable to get sugar into the cell, and a **glucose-trapping enzyme** that keeps sugar inside the cell, so the cell can burn it. Get it in quick, keep it there, and burn it. For the liver, which can do more than just burn glucose (liver is the only cell type that can liberate glucose from the cell), the mechanisms and enzyme kinetics must be different. But because all cells, including hepatocytes, have glycolysis as the default, we’ll explain how these specialized cells “add on” to the default mechanism. They don’t have DIFFERENT pathways, they have EXTRA pathways.

Glucose Transporters

Four glucose transporters get glucose into cells; two of them are important to know. GLUT1 and GLUT3 are very weak channels on every cell. Their affinity for glucose is extremely high, with K_M around 1 (low K_M = high affinity). But in enzyme kinetics, a high affinity does not necessarily mean a high velocity. These enzymes have a low V_{max} . They are the basal uptake channels. There are few of them, and they are mentioned here for completeness and to review K_M and V_{max} . Because Type I diabetics go into DKA without insulin, even though these basal channels are operational, we know that GLUT1 and GLUT3 aren’t enough.

TRANSPORTER	K_M (AFFINITY)	V_{MAX} (RATE)	TISSUE
GLUT1	3 (High affinity)	Super slow	Most all cells, basal uptake in all tissues
GLUT2	15 (Poor affinity)	Slow	Pancreas and liver (sense and release)
GLUT3	1 (Super affinity)	Super slow	Brain (brain gets priority for glucose)
GLUT4	5 (High affinity)	Really fast	Insulin-receptor cells, skeletal muscle, adipose

Table 3.1

So ignore GLUT1 and GLUT3 . . . but GLUT4 is for every cell, and GLUT2 is the exception transporter for the exception cells of the pancreas and liver.

GLUT4 transporters are the systemic default glucose transporter. They are regulated by **insulin** but are **NOT ligand-gated transporters**. They are the transporters that use passive diffusion but are only capable of action once they have been inserted into the plasma membrane. When insulin binds to its cellular receptor, **vesicular** GLUT4 transporters are inserted into the luminal wall of the cell. These have a K_M about five times that of the GLUT1 and GLUT3 transporters. That means GLUT4 channels actually have a much **weaker affinity** (higher K_M means lower affinity). This illustrates the power of affinity vs. V_{max} . Despite having a weaker affinity, GLUT4 transporters are the “best” or “fastest” glucose transporters we have—they have a **very high** V_{max} . And because insulin is only secreted by the pancreas as glucose levels rise, there is a favorable concentration gradient to get the glucose into the cell. Therefore, when these K_M -of-5 channels enter the membrane, their really fast V_{max} means glucose rapidly enters the cell. For most non-liver tissue, this makes sense—get the glucose in and start burning it.

Insulin (the ligand) binds the insulin receptor (cell membrane), and then vesicles loaded with GLUT4 insert into the plasma membrane of the cell, allowing glucose to rush into that cell.

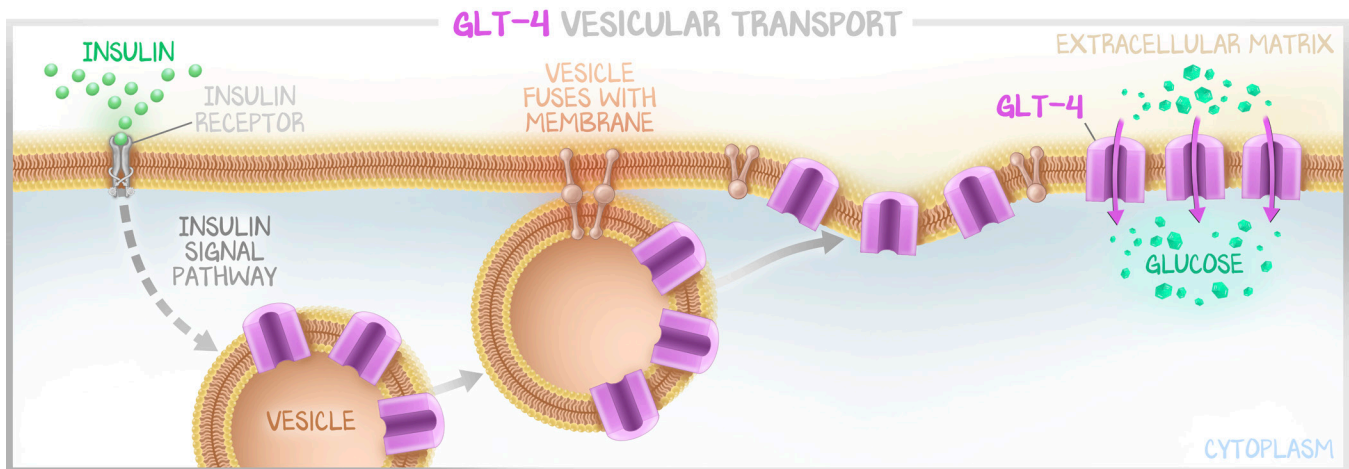


Figure 3.1: GLUT4 Vesicular Transport

Insulin binds to its receptor on the cell membrane. Insulin has many effects, but one of them is the insertion of GLUT4 receptors into the cell membrane. Vesicular transporters (GLUT4 channels in a lipid bilayer already in vesicles in the cytoplasm) fuse with the cell membrane, increasing the amount and density of passive diffusion of glucose

GLUT2 transporters are found on hepatocytes and β cells of the pancreas. The purpose of GLUT2 is quite different than GLUT4. The pancreas is responsible for sensing what the blood sugar is in the body, releasing insulin if it is too high and glucagon if it starts to dip low. That means it **can't** have glucose rush into the cell like GLUT4 would allow. And it certainly can't be insulin-receptor-dependent (the pancreas makes the insulin). So it would make sense that the pancreas would feel a high blood sugar only if the sugar got above a certain range, meaning it should be **insensitive to normal sugars**. Enzymatically, this is done via a **low affinity** and a **low V_{max}** . GLUT2 has a K_M of 15—high K_M , low affinity. A high K_M means that the enzyme requires a lot of substrate to work. As glucose (the substrate) levels rise, these channels start to work. What should happen is that these channels mark a rise in blood sugar, and so should induce insulin release from pancreatic cells, dropping the sugar back down below the concentration where these channels would work. And if the sugar gets a little high, the liver starts storing the sugar. This also makes sense for hepatocytes. While the hepatocytes will use dietary glucose while insulin is around, they are supposed to be able to **release glucose** when glucagon is around. That means it wouldn't make much sense if it had a transporter that brought glucose into the cell really well.

GLUT2 are low-affinity glucose sensors that only work when glucose is high, signaling the liver to store glucose and the pancreas to secrete insulin.

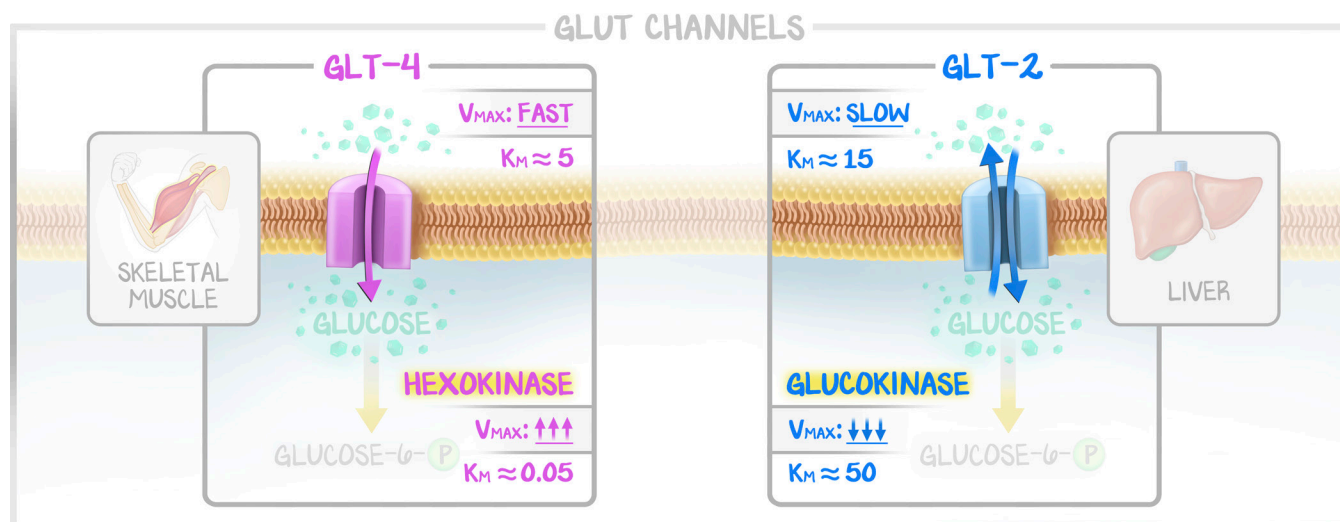


Figure 3.2: GLUT Channels

GLUT2 channels have a low affinity and low V_{max} , which mirrors the low affinity and low V_{max} of glucokinase in hepatocytes. These cells are designed to release glucose, so it makes sense that they have both a low-affinity glucose-uptake mechanism (GLUT2) and a low-affinity glucose-trapping mechanism (glucokinase). In comparison, skeletal muscle will need the glucose for contractions and will never release glucose into the bloodstream, thus the high affinity and high V_{max} of the GLUT4 transporter (uptake) and the hexokinase (phosphorylation-trapping).

Glucose-Trapping Kinases Mirror Glucose Transporter Features

Both **hexokinase** (all cells) and **glucokinase** (hepatocytes) trap glucose in the cytoplasm. The “trapping” is a product of **phosphorylation**. Phosphorylation, adding a phosphate to a molecule, is done by **kinases**. This step requires energy. Kinases take the energy from ATP, stick a phosphate onto the molecule, and leave the lower-energy ADP behind. As soon as glucose is phosphorylated, it cannot go through the glucose transporter and is, in effect, forever trapped in the cell. Because it is such a high-energy step, the phosphorylating of glucose is **irreversible**.

Hexokinase is that kinase in all cells. Just like GLUT4 has a moderate affinity and large V_{max} that favors uptake of glucose into the cell, so too does hexokinase have a strong affinity (K_M 0.05 in RBCs) for glucose, and a high V_{max} . Again, when the cell knows how to do one thing only—take glucose, use glucose—this makes sense. The transporter and the kinase both favor glucose staying in the cell and being used.

Glucokinase, however, is present in hepatocytes and pancreatic islet cells. Glucokinase has a low affinity for glucose (K_M 10) and a slow V_{max} . This is going to make a lot more sense when we learn how the liver does gluconeogenesis; any glucose the liver might make in the presence of hexokinase would be immediately trapped there. So glucokinase serves two purposes. First, like GLUT2, it acts as a glucose sensor, only active when glucose levels rise. Second, it allows hepatocytes the freedom to make glucose and not have it trapped in cells.

ALL CELLS			
	K_M	AFFINITY	V_{max}
GLUT4	5	↑	↑↑
Hexokinase	0.05	↑↑↑	↑↑

HEPATOCYTES			
	K_M	AFFINITY	V_{max}
GLUT2	15	↓	↓
Glucokinase	50	↓↓	↓

Table 3.2: Glucose Channels and Phosphorylators

Comparison of what most cells use (GLUT4 and hexokinase) and what the hepatocytes use (GLUT2 and glucokinase). Function drives enzyme kinetics, The liver wants to build and release glucose; other cells want to trap and use glucose.

Default Glycolysis: Don't Remember All the Details

We're going to walk through glycolysis, step by step. Our presentation **deemphasizes substrate names**, and instead focuses on what makes them similar to each other, or what happens at each step. We have also **removed non-essential enzymes—if the step is irreversible or energy-producing**, we pay attention, otherwise, worthless information. We have emphasized **irreversible steps** with a unidirectional red arrow, and we keep track of **energy** in green (ATP) and blue (NADH). There are so many “extras” to glycolysis because it's so important, it's nearly impossible to create a slide, a printed page, that isn't distracting. The video will scroll through the image part by part, as it is discussed. NOT everything happens at the same time. Let's go through the “normal” glycolysis, discussing the key points: what happens and what controls each step.

1. Glucose, a 6-carbon structure, enters the cell via a glucose transporter, and this first step is **irreversible and ATP-dependent**. A **kinase** adds a phosphate. Hexokinase/glucokinase (depending on the cell) phosphorylates glucose to create glucose-6-phosphate.
2. Glucose-6-phosphate is turned into fructose-6-phosphate.
3. Fructose-6-phosphate is **phosphorylated to 1,6-bisphosphofructose**. This step is **irreversible**, requires energy **from ATP**, and is the **rate-limiting step** of glycolysis. The kinase at work is **phosphofructokinase-1 (PFK-1)**.
4. The 1,6-bisphosphofructose gets chopped into two 3-carbon structures. Here is where things get confusing. Absolutely strictly, it gets chopped into two compounds: DHAP (which is used in fatty acids) and **glyceraldehyde-3-phosphate**. But because there is an isomerase between glyc-3-p and DHAP, for the purposes of glycolysis, there are in fact **two glyc-3-p**. It's this “two” bit that gets people. We had a 6-carbon structure, fructose, and chopped it in half, to make two 3-carbon structures. Since the two 3-carbon sugars are essentially the same thing, we account for all the carbons by going through the next part of glycolysis “twice for each glucose,” or “twice for each 6-carbon structure that entered at the very beginning,” because “two 3-carbon sugars came from

the one 6-carbon.” If that sounds confusing, everything that happens after the dashed line (on the whiteboard in the video) will have a $\times 2$ next to it.

5. **Glyceraldehyde-3-phosphate dehydrogenase** is the enzyme that will create a high-energy bond. It’s still reversible. It will take an uncharged, low-energy NAD and make a **high-energy NADH**. At the same time, it **phosphorylates** glyc-3-p to 1,3-bisphosphoglycerate (**1,3BPG**). This generates an NADH molecule which will be sent to the electron transport chain.
6. **1,3BPG** gives rise to **3-phosphoglycerate, generating an ATP**.
 - a. This ATP generation is not electron transport chain-dependent, and so is called **substrate-level generation**. This is the first time energy has been harnessed to make ATP. For every glucose (6-carbon) there are two 1,3BPGs (3-carbon) and every 1,3BPG gives one ATP, so every glucose molecule gives two ATPs. The system has just recuperated its ATP.
 - b. **Phosphoglycerate kinase** is the enzyme that does this reaction. Kinases are supposed to add a phosphate, but in this reaction we just removed a phosphate from the substrate and added it to an ADP to make ATP.
 - c. In the direction of forward-for-glycolysis this enzyme is named wrong. Kinases should add phosphates. But since this reaction is reversible, the same enzyme can go backward-for-glycolysis, and is therefore named correctly. Backward-for-glycolysis is forward-for-gluconeogenesis. Since each reversible reaction can have only one enzyme named, it has the name it has.
7. 3-phosphoglycerate to 2-phosphoglycerate via mutase, then 2-phosphoglycerate to phosphoenolpyruvate (PEP) by enolase. Throw-away substrates, throw-away enzymes. Don’t remember them.
8. Phosphoenolpyruvate, by means of **pyruvate kinase**, undergoes an **irreversible reaction** that **generates ATP** to form **pyruvate**. Similar to #6, but with stark contrasts listed in the following three subpoints, the enzyme is named “incorrectly,” and is another example of substrate-level phosphorylation.
 - a. A kinase should add a phosphate to the substrate, so pyruvate kinase, the way it’s named, should add a phosphate to pyruvate to make PEP.
 - b. However, unlike the 1,3BPG to 3-phosphoglycerate step, PEP to pyruvate is **irreversible**.
 - c. Pyruvate kinase **NEVER** becomes PEP in humans. But chemically, we discovered the enzyme in the pyruvate to PEP direction, so that’s how it got its name.
9. The generation of one pyruvate (3-carbon) gives us 1 ATP, so that for every one glucose (6-carbon), 2 pyruvates (3-carbon) undergo this process, for a **gain of 2 ATPs** per glucose molecule.

For medical biochemistry, memorize that the two steps of glycolysis that yield substrate-level phosphorylation are both kinases and are both named backward.

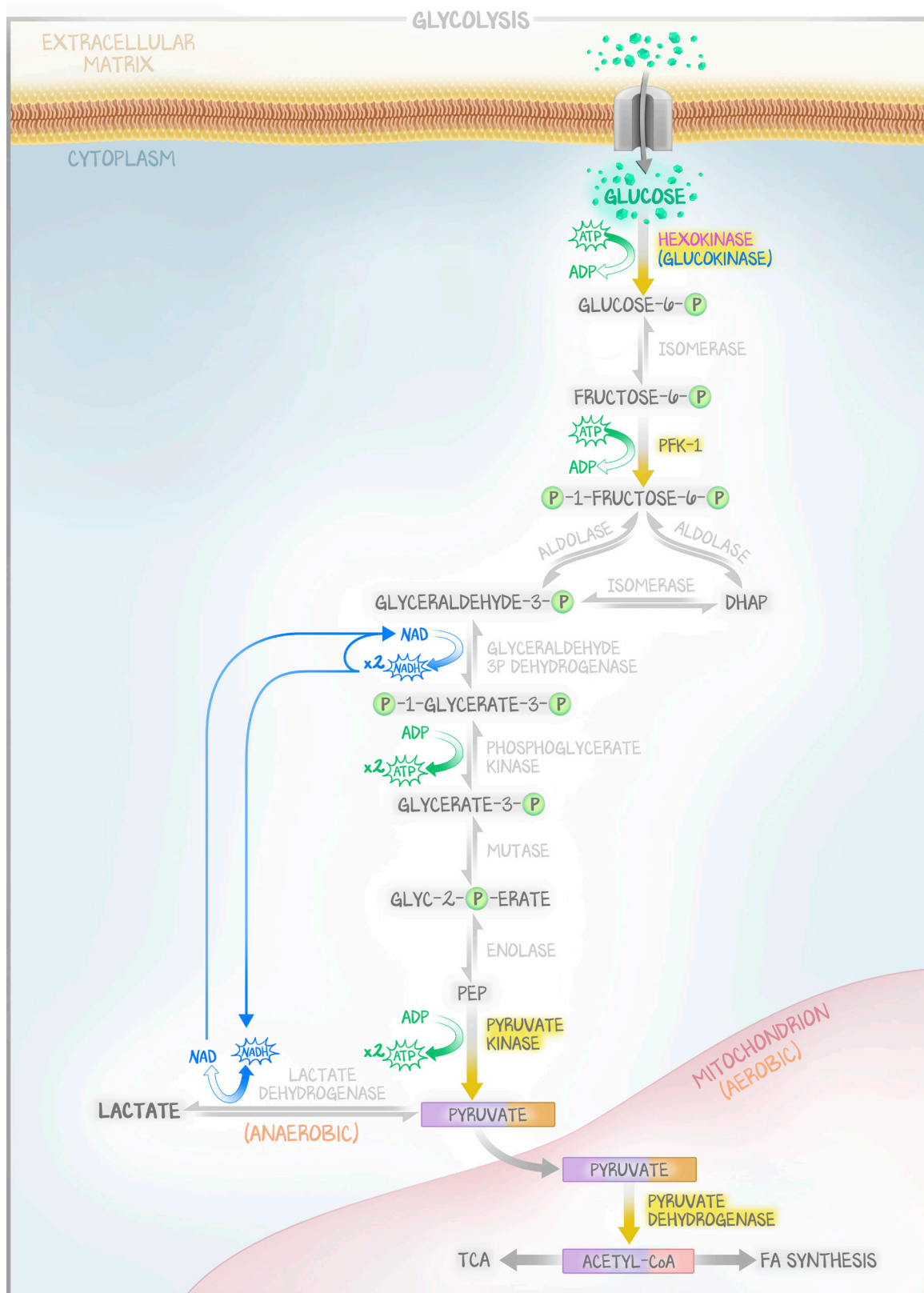


Figure 3.3: Glycolysis

The complete glycolysis pathway, with every detail and enzyme name. You will NOT MEMORIZE THIS. It's included for completeness. We address the red arrows in great detail. The red arrows are the irreversible steps, and the only steps that matter for medical biochemistry.

The Energy Count

At the end of glycolysis, we have a pyruvate in the cytoplasm, two NADHs and a net two ATPs. Glycolysis produced four ATPs. Glycolysis used two ATPs. Glycolysis produces a net of two ATPs.

Glycolysis Rate Control in Hepatocytes

A hepatocyte does the same thing as every cell in the insulin-dominant state. It takes dietary sugar, then uses dietary sugar. Hepatocytes use GLUT2 and glucokinase, but everything else is the same. **PFK-1** is the **rate-limiting step** in glycolysis. But hepatocytes might want to go backward (gluconeogenesis). So, the master creator saw that there was already code for glycolysis everywhere, and decided that it would be easier, rather than rewrite glucose metabolism for the hepatocyte, to **add regulation** to the existing default system.

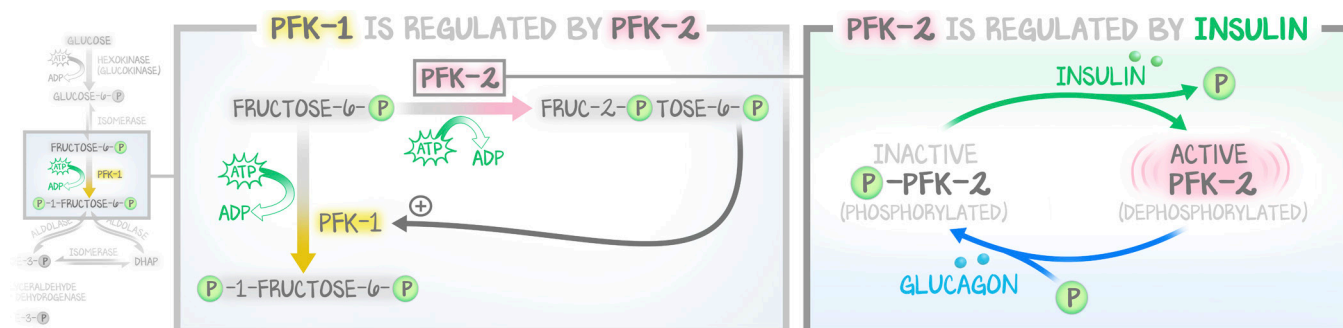


Figure 3.4: Regulation of PFK-1

PFK-1 is regulated by the product of PFK-2. PFK-2 is regulated by hormonal influence: insulin dephosphorylates PFK-2 to its active form, and glucagon phosphorylates it to the inactive form.

When glucose levels rise, in the **insulin-dominant state**, the liver should be burning glucose. That is, when sugar is abundant, insulin should tell the hepatocyte to act like all the other cells and burn through glucose. But when insulin is NOT around, in the fasting state, in the **glucagon-dominant state**, the liver should be **making glucose, not using it**. And if PFK-1 is the rate-limiting step of glycolysis, you can bet insulin and glucagon are going to fight over its activity (there is more to it than this, of course, but this is so complicated and so important that it comes up in multiple lessons, starting here).

Insulin will **dephosphorylate** an enzyme called **PFK-2**. By dephosphorylating PFK-2, it activates it. **Glucagon**, the hormone of the starved state, insulin's opponent, **phosphorylates PFK-2**, thereby **deactivating it**. This is an illustration where phosphorylation DOES NOT mean "activation," only "change." Insulin dephosphorylates PFK-2, activating it, causing PFK-2 to make **fructose-2,6-bisphosphate (F-2,6-P₂)**. **F-2,6-P₂** is a substrate that binds to and stimulates PFK-1. Insulin stimulates PFK-1 indirectly by dephosphorylating PFK-2; glucagon inhibits PFK-1 indirectly by phosphorylating PFK-2.

Aerobic Metabolism

When there is oxygen, pyruvate goes on to the mitochondria with the help of oxygen. The **NADH** that was made during glycolysis will be separately dispatched to the electron transport chain (at least the energy it contains will). When NADH runs the ETC, it generates **three ATPs**. I personally don't like counting my ATPs until they've converted, so at the end of glycolysis, in the presence of oxygen, we've got a **net of two NADHs** and a **net of two ATPs**.

Anaerobic Metabolism

When pyruvate kinase turns PEP into pyruvate, in the **absence of oxygen**, pyruvate can't go to the mitochondria and do its thing. In the absence of oxygen, there can be no electron transport chain. So what are we going to do with these two NADHs and this pyruvate? Well, if all that happened was that NAD became NADH, we'd quickly run out of NAD. So, this last step **regenerates NAD from NADH** but **without generating ATP**. That at least gives back the NAD to go through glycolysis again.

That reaction requires pyruvate to make **lactate**. Lactic acid is a byproduct of cellular respiration, glycolysis, in the **absence of oxygen**. It is a clinical marker for hypoperfusion during shock—the tissues still trying to do their thing without oxygen. Lactate is also what causes muscle pain while running or lifting weights—muscles pushed past the point of oxygen delivery accumulate lactate. So, too, do any cells not properly perfused.

However, **lactate dissipates quickly** when oxygen is restored. The link between lactate and pyruvate is reversible.

Red Blood Cells Have No Mitochondria

What happens if there's plenty of oxygen (like a red blood cell loaded with oxygen on hemoglobin), but no mitochondria? The electron transport chain requires both oxygen and a mitochondrion. So...if there are no mitochondria, the cell must use anaerobic metabolism—pyruvate to lactate.

The **Cori cycle** is never discussed in this series. Effectively, if RBCs only burned glucose to create lactate, lactate would accumulate. The Cori cycle is a way the red blood cells regenerate their NAD (pyruvate to lactate) but then eliminate the lactate from their cytoplasm. Know that it exists.

The influence of 2,3BPG on RBCs is quite important. It's an alternative pathway in glycolysis used only by the RBC. However, metabolically it is irrelevant, and we discuss **2,3BPG in Hematology**. Dealing with it here would only cause confusion.